are believed to be contributed by the rotational motion of the side methyl groups [3]. Distinguishable dynamical behavior found between two proteins reveals local flexibility and conformational substates unique to oligomeric structures. Our results greatly help understanding the relation between protein dynamics and their biological functions.

[1] X.-Q. Chu, et al, JPCB. 116, 9917 (2012).
[2] X.-Q. Chu, et al, Soft Matter 6, 2623 (2010).
[3] X.-Q. Chu, et al, JPCL. 4, 936 (2013).

3289-Pos Board B17

Allosteric Regulation of Protein Kinase Enzymes via an Electrostatic Switch that Modulates Active Site Dynamics

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Protein kinase (PK) enzymes are a large family of signaling proteins that play a central role in signal transduction pathways. Robust regulation of their catalytic activity is critical, and many oncogenes harbor mutations that result in misregulated PK activity. The chemical basis for how some PK regulatory factors ultimately affect the rate of chemistry is still not completely understood. We have identified a long-range electrostatic switch that we believe is used by allosteric PK regulatory factors to modulate the rate of chemistry by tuning active-site dynamics.

We applied a combination of crystallography, kinetics, and molecular dynamics to determine the chemical kinetic basis for how this electrostatic switch, toggled by regulatory subunit binding, affects each step of catalysis by CDK2 kinase. We engineered point mutants to deconstruct the kinetic, dynamic, and thermodynamic consequences of the switch. We also evaluated other PKs and find that, although it has evolved to be triggered in different ways by diverse PK regulatory factors, the mechanics of this switch can be conserved.

We demonstrate that a key component of the switch is that it affects a significant change in the electrostatic potential within the ATP*Mg binding site of the enzyme. This electrostatic effect is propagated through the low-dielectric protein interior and directly affects the two dominant rate-determining steps of catalysis: attenuating both the recruitment of catalytically essential Mg cofactors (affecting both kcat and KM) as well as the release of the ADP product.

Conclusion: We present a chemical hypothesis that provides a mechanistic explanation for one way that a large-scale conformation transition, observed in diverse PK family members, is able to significantly affect the rate of chemistry by acting at a distance from the active site.

3290-Pos Board B18

A Network of "Molecular-Switches" Control the Activation of Key Bacterial Signaling Protein

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Recent successes in simulating protein structure and folding dynamics have demonstrated the power of molecular dynamics to predict the long timescale behavior of proteins. Here, we extend and improve these methods to predict conformational change pathways between active and inactive state of nitrogen regulatory protein C (NtrC). By employing unbiased Markov State Model based molecular dynamics simulations, we predict a new dynamic picture of the activation of a key bacterial signaling protein NtrC, involving a complex network of molecular switches. These results are consistent with experimental observations and predict new mutants that could be used for validation of the mechanism. Moreover, these results suggest a novel mechanistic paradigm for conformational switching.

3291-Pos Board B19

Coarse-Grained Modeling of the Dynamics and Allosteric Modulation of Hras Protein

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HRAS acts as molecular switch by cycling between active (GTP-bound) and inactive (GDP-bound) state during signal transduction processes associated with cell growth and differentiation. The wealth of biochemical and structural data available for HRAS has identified critical regions in protein structure that plays crucial role in signalling. However, the mechanism by which active and inactive state transition occurs is not yet completely understood due to lack of experimentally determined intermediate structures. Also, the timescale at which these processes occur is currently beyond the reach of all-atom molecular dynamics simulations. In this talk, I will describe the dynamics of GDP and GTP bound form of HRAS with and without GEFs, using a transferable intermediate resolution model developed by us. In the model, the backbone is represented with atomic resolution but the sidechain with single bead and it has sufficient predictive power so that– Starting from random initial configurations, the model properly folded 19 proteins (including a mutant sequence) in to native states containing β-sheet, α-helices and mixed α/β. The model is then used to predict the dynamics of HRAS. The predictions of the coarse-grained model are tested with different 100 ns simulations. We present intermediate states and demonstrate, among other results, that the opening of Switch I/β-2 region in HRAS-GTP is a thermally activated process and occurs in the absence of GEF.

3292-Pos Board B20

Redistribution of Flexibility in Stabilizing Antibody Fragment Mutants Follows Le Chatelier's Principle

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Proteins exhibit rich dynamic behavior, yet protein structure is highly constrained by cross-linking H-bonds. This dichotomy makes it difficult to predict the effect of individual mutations on protein thermodynamics and dynamics. For example, it is often perceived that stabilizing mutations cause a protein to become more rigid due to improved packing. However, due to the complex relationships between rigidity and thermodynamics, mutations can cause unexpected long-range effects such as entropic stabilization in conjunction with increased dynamics. In general, mutations shift the rigidity-flexibility equilibrium within the native state ensemble. We quantify these shifts across a handful of stabilizing mutants within the single chain F_v (scFv) anti-lymphotoxin-β receptor antibody fragment using all atom explicit solvent molecular dynamics simulation, where the MD trajectories over 100 ns are analyzed by a Distance Constraint Model. Despite all of the mutants being stabilizing, we observe a statistically significant redistribution of rigidity/flexibility that extends far from the mutation. For the scFv fragments, we find that an increase in flexibility is much more common than an increase in rigidity. Interestingly, a net gain or loss in flexibility of an individual mutant is typically skewed. The redistribution of flexibility can be mechanistically traced to changes in the H-bond network. For example, the formation of new H-bonds due to a stabilizing mutation will generally induce a local increase in rigidity, while at the same time H-bonds break elsewhere, causing a frequent increase in flexibility far removed from the mutation site. Increased flexibility within the VH β-four and β-five loops is a noteworthy illustration of this long-range effect. As a general rule of thumb, we suggest that rigidity-flexibility equilibrium shifts manifest themselves through enthalpy/entropy compensation in the native state ensemble as the protein structure adjusts via Le Châtelier's principle.

3293-Pos Board B21

Generalized Model-Free Spectral Density Analysis Applied to Rhodopsin Activation in Membranes

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Although molecular structures of G-protein-coupled receptors (GPCRs) are becoming increasing available from X-ray crystallography, understanding their functions requires information about molecular dynamics in membranes. Here we use rhodopsin as a model to illuminate general features of GPCR activation. With solid-state ^{2}H NMR spectroscopy we obtain experimental data pertinent to both structure and dynamics. Experimentally, order parameters and relaxation rates are the two observables of solid-state ^{2}H NMR experiments. We propose that the local dynamics of the retinylidene ligand are coupled to large-scale fluctuations of the transmembrane helices of rhodopsin, leading to activation of the receptor. To study the structural dynamics of retinal bound to rhodopsin, we start with an irreducible representation of the correlation function in terms of mean-squared amplitudes and correlation times [1]. The mean-squared amplitudes are related to the orientational order parameter, while the irreducible correlation times include the preexponential factor and energy barrier. To bridge the generalized model-free theory with experimental measurements, we separated the relaxation rates into spectral densities by applying Redfield theory. The spectral

densities are Fourier transformation partners of the irreducible correlation functions. By fitting theoretical spectral densities to experimental data we can readily obtain the values of preexponential factors and activation energies [2]. We are currently applying our generalized model-free method to interpret the behavior of active Meta-II rhodopsin. Our aim is to establish if the local fluctuations of the ligand initiate the structural changes of rhodopsin to understand the activation mechanisms of GPCRs in general. Moreover, the results from our generalized model-free analysis method can be used in molecular dynamics (MD) simulations without the limitations of simplified motional models. [1] M.F. Brown (1982) *JCP* **77**, 1576-1599. [2] A.V. Struts *et al*. (2011) *NSMB* **18**, 392-394.

3294-Pos Board B22

Conformational Motion in Gene Regulatory Proteins

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Proteins' structures determine functions and the functions determine the structures. Yet, proteins and other biological macromolecules are dynamic systems under continuous conformational motion and the motions are keys to many of the biological processes in which they are involved. We argue that conformational motion driven by structural specificities, and not structures alone, determines biological functions. For instance conformational motion in gene regulatory proteins acts as a selective molecular switch motion in DNA binding mode and controls gene regulations. Perhaps, such action is relatively well studied in prokaryotic OxyR belonging to LysR family of transcriptional regulatory proteins. Escherichia coli Oxidative stress response genes are transcriptionally regulated by OxyR through a reversibly reducible cysteine disulfide biosensor. The redox status in these cysteines induces structural changes which are conformationally transmitted to the dimer subunit interfaces and alters DNA binding mode. However, crystal structures of Porphyromonas gingivalis OxyR regulatory domains indicate locked dimer configuration insensitive to cysteine disulfide redox status and shows only one activating mode. Conformational motion in Porphyromonas gingivalis OxyR changes dimer/tetramer convention (dimer binding to DNA or tetramer binding to DNA) and alters differentiation in gene regulations. Crystal structures along with modeled full-length Porphyromonas gingivalis, Escherichia coli and Neisseria meningitidis OxyR-DNA complexes predict different DNA binding modes in these organisms and indicate how the limited conformational motion differentiates the species. Aerobic organisms' OxyR is confined in open dimer configuration; Anaerobe's OxyR is confined in closed dimer form, while facultative organisms can conformationally switch OxyR dimer configurations. Conformational motion in aerobes and anaerobes OxyR is restricted by dimer configuration and can only change dimer/tetramer population while in facultative organisms conformational motion induces configurational switch in dimers.

3295-Pos Board B23

Differences in Troponin C Dynamics Between Cardiac and Skeletal Muscle - A Molecular Dynamics Perspective

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Troponin (Tn), part of the thin filament in cardiac as well as skeletal muscle, plays an important role in calcium signaling events in muscle contraction. It acts as a Ca^{2+}-dependent switch, activating and deactivating the myofilament leading to contraction and relaxation of the muscle cell. An important mechanism in the regulation of contraction is the opening up of the Troponin C (TnC) hydrophobic patch to allow Troponin I (TnI) to bind. Two main structural differences are observed between cardiac TnC (cTnC) and skeletal TnC (sTnC): sTnC binds two calcium ions in its N-terminal regulatory domain, while cTnC only binds one calcium ion. The calcium binding site I in cTnC is inactive due to mutations. Additionally, the hydrophobic patch between helices A and B in the N-terminal regulatory domain is open in sTnC, while it is closed in cTnC. In previous work we performed microsecond molecular dynamics (MD) simulations of cTnC in different states of calcium binding to estimate the free energy difference for opening of the hydrophobic patch. Here we present long time-scale MD simulations of sTnC in its apo, one Ca^{2+}-bound and two Ca^{2+}-bound forms. We observe a 10,000-fold increased opening frequency in sTnC compared to cTnC. The dramatic difference is caused almost entirely by changes in the binding site I dynamics upon calcium binding. Investigations of sTnC mutations shown to prevent calcium binding to site I shed further light on the differences between the TnC isoforms. Additionally, Brownian dynamics simulations are used to investigate TnI association with TnC. Simulations of a full length troponin model elucidate the dynamical interplay between the TnC, TnI and TnT subunits.

3296-Pos Board B24

The Role of Conformational Flexibility in Inhibitor Binding and Substrate Recognition for Cyp119

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Conformational flexibility of several cytochromes P450 has been observed in response to substrate and ligand binding, and thus may play an important role in catalysis. CYP119, a bacterial thermophilic protein from cytochrome P450 superfamily has been observed in three different conformations with different inhibitors bound using X-ray crystallography, but the significance of these states in solution and in the function of the enzyme is not well known. According to the crystal structures, much of the diversity in conformational states arises in F and G helices, which fold around the substrate binding site at the distal heme face. Double electron electron resonance (DEER) was used to measure the average distances and the distance distributions between spin-labels for populated conformational states in solution. Pairs of spin-labels were introduced by coupling to engineered cysteines on the protein surface, and the effects of labeling on ligand dissociation constants (K_d) and enzyme function were characterized. DEER results from three different mutants of CYP119 indicated that 4-Phenylimidazole binding results in a conformational state that is distinct substrate-free and Imidazole-bound forms. The DEER distance changes between the two conformational states were determined as 10Å for two of the mutants and 12Å for the other one. Data will also be presented for the complex between CYP119 its proposed substrate, lauric acid. These results will be discussed in terms of models for substrate recognition and gated functional behavior in these enzymes.

3297-Pos Board B25

Conformational Changes in Protein Binding Processes

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Ligand-protein and protein-protein binding processes play a crucial role in biological systems. They are often associated with conformational changes that induce effects such as signal transmission or allostery. To structurally and energetically explore the interplay between induced fit, conformational selection, and allostery, we performed molecular dynamics simulations of three selected proteins.

As a first system, we choose the cAMP binding process at the potassium channel MloK1. The obtained free energy differences between the two main protein conformations, an open and a closed state, reveal that the process is best described by an induced fit mechanism. We found that the binding affinity is mainly caused by the conformational change.

Next, we explored the structural determinants of allostery of the export protein CRM1. This ring-shaped protein plays a crucial role in the nucleocytoplasmic transport of macromolecules. We investigated what structural features and how the binding of RanGTP and cargo proteins determine the overall conformation. We found that the enforced rearrangement of a key helix due to RanGTP binding changes the stability of the overall conformation. This induces a global conformational change, which in turn causes a local conformational change in the cargo binding site. The link between global and local conformation leads to cooperative binding.

Third, we investigated the influence of ligand binding on the dimerisation of nitrate reductase. This enzyme is a key player in nitrogen fixation and binds a molybdenum containing cofactor in its active centre. We identified key motions caused by the cofactor binding, and suggest how these motions might be coupled to dimerisation.

Overall our simulations underline and explain how subtle free energy changes due to ligand/protein binding can change the overall protein free energy landscape thus causing conformational changes, which are key to the protein function.

3298-Pos Board B26

A Model for Allosteric Control of Pore Opening by Substrate Binding in the Eutl Microcompartment Shell Protein

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The ethanolamine utilization microcompartment (eut MCP) is a giant protein assembly that acts as a metabolic organelle in enteropathogenic bacteria, allowing them to proliferate in the human gut. The eut MCP consists of a polyhedral shell, reminiscent of a viral capsid, which encapsulates several sequentially